PERMANENT PREPARATIONS OF TISSUES AND ORGANS TO SHOW GLYCOGEN

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From the time of Claude Bernard's discovery of glycogen (1850-1860) it has been known that strong alcohol retains it in the tissues, and therefore alcohol has been used whenever one wished to determine the presence of this substance by microscopic examination. Absolute alcohol is usually recommended for the fixation, but as glycogen does not dissolve in alcohol above 60% it seems unnecessary to use absolute. 95% alcohol has given perfectly satisfactory results. Some authors recommend formalin and other fixers. It is true that some glycogen remains and can be demonstrated after the use of formalin and some other fixers; but all are agreed that alcohol is the most satisfactory fixer when glycogen is to be demonstrated. As alcohol penetrates slowly one can get the best fixation by using only small or thin pieces suspended in the alcohol. Bernard himself recommended the use of a mixture of equal parts of a saturated alcoholic solution of iodin and glacial acetic acid prepared at the time of use. This mixture is not so satisfactory as alcohol alone. A modification of Bernard's method has been found excellent, and it has the advantage of not shriveling the tissues so much as pure alcohol. The formula is 95% alcohol, 100 cc.; 10% iodin in 95% alcohol, 2.5 cc.; glacial acetic acid, 1 cc.

For sectioning, the paraffin method is the most satisfactory. The sections are spread on slides, using one of the following iodin solution instead of water: Water, 500 cc.; sodium chlorid, 1.5 grams; potassium iodid, 3 grams; iodin crystals, 1.5 grams (or 15 cc. of a 10% solution of iodin in 95% alcohol). If one has to do with very soluble forms of glycogen an alcoholic solution for spreading the sections is preferable. Formula: Water, 250 cc.; 95% alcohol, 250 cc.; sodium chlorid, 1.5 grams; potassium iodid, 3 grams; iodin crystals, 1.5 grams.

When the sections are spread care must be taken not to overheat the slides, for the *paraffin should in no case be melted.* While the

sections are being spread the iodin stains the glycogen, and one can examine the sections with low powers without further treatment. The stain remains in the paraffin sections for an indefinite period. This gave a clue, for permanent preparations of iodin-stained glycogen. The ordinary hard paraffin shows crystals too plainly. It was thought that if a liquid or semi-liquid paraffin could be used it might, like the solid paraffin, preserve the iodin stain in the glycogen. In sections so mounted high powers of the microscope could be used.

Many forms of liquid paraffin were tried, but none of them were satisfactory. White vaseline was then used at the suggestion of Dr. Mall, and it answered fairly well. The best results were, however, obtained by the use of the ordinary yellow vaseline sold by druggists. After many experiments the final mounting was accomplished as follows: If the paraffin sections had faded or were not deeply enough stained the slide bearing the sections was immersed in one of the staining solutions given above for 5 or 10 minutes or longer. The glycogen will be deeply stained if the paraffin has not been melted in spreading the sections. The slide is then allowed to dry thoroughly—half an hour in a drying oven or an hour or more (preferably over night) in the laboratory. The yellow vaseline is melted over a water-bath, and then the slide with the sections is put into xylene until the paraffin is dissolved out of the sections —2 to 5 minutes. After the paraffin is removed the sections are mounted in the yellow vaseline as one mounts preparations in balsam. The slide may be slightly warmed so that the vaseline can be mostly pressed out; the excess of vaseline is wiped away and the cover sealed with balsam or shellac, so that it will not be easily moved. Preparations so made have retained the glycogen stain of iodin for three years.

Another method was tried with success, *viz.*, that of mounting in balsam without a cover-glass as with Golgi preparations. Preparations so made have retained the stain for over six months. The balsam preparations are somewhat more satisfactory for study with the highest powers.

If homogeneous immersion objectives are to be used on these uncovered preparations it is better to revert to the original homogeneous immersion liquid, *viz.*, thin Canada balsam (See Cox, Proc.

Amer. Micr. Soc., 1884; Mayall, p. 96). Of course, the ordinary cedar oil may be used on the covered preparations mounted in vaseline. It will be found in most, if not in all, of the tissues which were quickly and thoroughly fixed that the glycogen is by no means in granules, but appears as a homogeneous substance in the cells.

Many attempts have been made to use other stains than iodin for the glycogen, that is, stains which would be permanent and enable the investigator to mount the sections in balsam. None of the methods so far proposed give so differential a stain as iodin; and they are difficult of application. They are of course desirable to use in connection with the iodin stain if one is making a critical study.

The two methods most often employed are the gentian violet method of Lubarsch and the carmin method of Best. Both these methods are quite fully and satisfactorily dealt with in Ehrlich's Encyclopedia of Mikroskopie, under Glycogen.

In working upon embryonic and some other material it is sometimes desirable to decalcify the contained bone. This can be safely done, as follows: Fix thoroughly in the absolute or 95% alcohol or the iodin alcohol given above. Decalcify in 67% alcohol containing 3% nitric acid (Gage, Proc. Amer. Micr. Soc., 1892, p. 121). The sections are made by the paraffin method as before. Of course, one may use the collodion method of sectioning, but then the only way known to the writer for making permanent preparations stained with iodin is to allow the collodion sections to dry. They may then be mounted in vaseline or in balsam as directed above.

If one attempts to dehydrate the sections the iodin will be dissolved out. The secret of success is to stain the sections before the removal of the paraffin; to use no alcoholic solutions after the sections are stained with iodin, but to mount directly in vaseline and cover, or in balsam without a cover-glass, as directed above.